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Detection of Biofilm Formation for Aerobic Bacteria Isolated from Burn and their Antibiotics Resistance in Hospitals in Ramadi City

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Abstract
A total of 86 samples of burn swabs were collected from patients between the ages of (16-56 years).All samples were tested on several culture media. The sensitivity of the isolates to antibiotics was tested by using disc diffusion method. The results of this study showed that the most pathogenic cause of burn injuries was*Pseudomonas aeruginosa*25(29.07%) and Then *E.coli*19(22.09%), *Staphylococcusaureus*18(20.93%), *Proteus mirabilis*10(11.62%), Coagulase-negative Staphylococci8(9.32%), *Klebsiella pneumonia* 6(6.97%) respectively. In the sensitivity test to antibiotics, it was noted that both*Staphylococcusaureus* and Coagulase negative *Staphylococci*a high was sensitive Vancomycin,Cefepimeand Imipenem. In the study, it was observed that all gram-negative bacteria were sensitive to the antibiotics Imipenem,Cefepimeand Tobramycin. It has a different resistance to other antibioticsThe study aimed to know the most prevalent bacteria in burn injuries, their sensitivity to antibiotics, and their ability to form a biofilmin hospitalize patients Ramadi city
Key words: *p. aeruginosa*, Burn injury, Burn infection, Biofilm

Introduction

Burn infection considered one of important medical danger , there are many reasons could lead for it, such as heat, chemical agent, electricity... etc. , The large problem in burn is the infection when the skin is destroyed, many pathogens and opportunistic microbes are initiated the infection and this lead to sepsis [1,2].It is one of the main causes of high morbidity and mortality in hospitalized burn patients [3]. the patients with burn injury must take essential care to prevent mortality [4].The multi-drug resistant Gram-positive bacteria and Gram-negative bacteria is used to the most microorganisms linked to injuries infection, according to many studies, these microorganisms include *Staphylococcus aureus* and *Pseudomonas aeruginosa* and other microorganisms like *Escherichia coli*, *Klebsiella pneumonia* and *Acinetobacter spp*. consider as a nosocomial pathogens[5,6]. After wounds contamination and adherence, these microbes begin to penetrate the viable tissue and invasive it, invasiveness of microbes is depend on the size of local wound and who the patients' health [7].The study aimed to know the most prevalent bacteria in burn injuries, their sensitivity to antibiotics, and their ability to form a biofilmin hospitalize patients Ramadi city.

Material and Methods

Collection of Samples

This study have collection about 88 samples (65 males and 23 females) which collected from patients with burn injury by assistance of physician throughout the period fromJanuary 2021 to March 2021, from AL-Ramadi Teaching Hospital. the patients ages were 16 to 56 years old. Samples were obtained by utilizing sterile cotton swaps with sterile normal saline, they put in to brain heart infusion broth. and directly transported to the lab and then cultured in to different media for microbiology diagnosis that was achieved according to [8,9]

Identification of aerobic microorganism

Different bacterial isolates were characterized by several methods:

Morphological examination was performed with Gram stain and cultured bacteria on the selective media includedNutrient agar, Blood agar, Maccokeyagar ,mannitol salt agar and incubation was done for 24 at 37 °C.A series of biochemical tests were also carried out including: IMVC, urease, coagulase ,oxidase and catalase tests for diagnosing confirmed of the isolates were performed vitek-2 system.

The same is true for *P. aeruginosa* bacteria whose ability to produce pyocyanine and protease was tested by cultured on agar of cetrimide andskim milk agarrespectively.

Antibiotics Test

The sensitivity of the isolates to antibiotics was tested by using disc diffusion method(10). The pure colony for each bacteria was streaked onto Muellur Hinton agar . For gram positive bacteria, five different antibiotics disc were used which provided from Oxoid-USA, these antibiotics are Amoxicillin/ Clavulanic acid (20/10 µg), Amikacin (30 µg), Vancomycin (30µg), Imipenem (50µg) and Cefepime (10µg). While for gram negative bacteria, five different antibiotics disc , these antibiotics areAmikacin (30µg), Cefotaxime (30µg), Imipenem (10µg), Cefepime (10µg) and Tobramycin (10µg). The sensitivity and resistance of bacteria was determined based on the inhibition zone after period 18-24h from culture. The inhibition zone that form was compared with CLSI 2017[11].

Biofilm formation assay

The procedure that was adopted in this study was performed according to [12] by the wells of sterile 96 well U shaped –bottomed polystyrene microplates .

Statistical analysis

The data that obtained were displayed as mean ± SD and statistical significances were measured utilizing test of ANOVA[13].

Results and Discussion

Identification of Microorganisms in burn injury

In this study included 86 burn injury patients. six different microorganisms were isolated in this study. Twentyfive (29.07%) patients swabs were gram negative bacteria *p.aeruginosa* ,nineteen (22.09%) for *E.coli* ,ten (11.62%) for *P. mirabilis*, six(6.97%) for *K.pneumonia* while Gram positive bacteria *s. aureus*Eighteen (20.93. %) and Coagulase-negative Staphylococci eight (9.32%), swabs

patients. as showed in Table 1. These results were in agreement with by Aljanaby et al, they found in their study that The most common bacteria was *P. aeruginosa* (27.6%)[14]. *P.aeruginosa* has ability to produce infection because the high rate of virulence and antimicrobial resistance[15]. Both *P. aeruginosa* and *E.coli* have high diffusion rate and consider as a nosocomial pathogens in admitted Hospitalize patients. Another bacteria such as*s.aureus* , *P.mirabilis* , K pneumonia were isolated and this result are in agreement with previous studies[16,17]. Results showed the most isolated bacteria which has multi-antibiotics, resistance is *P. aeruginosa* and followed by *E.coli* and *S. aureus*,*P. vulgaris*. this either could belong to their ability to acquire the genes that responsible for drug resistance from other bacteria in the environment or belong to ability of Gram negative bacteria especially *P. aeruginosa* to form biofilm that protect bacteria from the effect of host immunity and antibiotics[18,19]. *P. aeruginosa* is one of the most important pathogens causing different infections such as bacteremia and burn infection[20]. also has more than 70% mortality of Burn infection[21]

Antibiotics susceptibility

The results of the current study showed that Gram positive bacteria *S.aureus* Showed a resistance against Amoxicillin/clavulanic acid,AmikacinAt 36% and 20% respectivelyand is was a high sensitivity against Vancomycin,Cefepimeand Imipenem.as showed in (Table 2).Coagulase negative Staphylococci showed it was It is sensitive to all antibiotics exceptAmoxicillin/clavulanic acid.Gram Negative Bacteria *P.aeruginosa* showed resistance against Cefotaxime,AmikacinAt 34% and 20% respectively,Sensitive to other types of antibioticsAs showed in(Table 3).The same is true for the bacteria *E. coli* showed a resistance against Cefotaxime, Amikacin At 26% and 23% respectivelySensitive to other types of antibiotics*P.mirabilis* showed a resistance against, Cefotaxime, Amikacin At 24% and 22% respectivelySensitive to other types of antibiotics..*K.pneumonia* showed resistance against, Cefotaxime, Amikacin At 36% and 24% respectivelySensitive to other types of antibioticsas in Table 3.

Biofilm assay

The current study findings highlighted biofilm formation by isolates isolated from burns. Table No. 4 shows that *P. aeruginosa*isolates were have strongly biofilm more than any isolate.It possessed sixteen strong biofilm formation isolates out of a total of 25 isolates of *P. aeruginosa*, and thus it is the most isolate producing a strong biofilm from the remaining isolates.

Table(1) Isolates, their number and percentage

The bacteria	No. of bacteria	Percentage
Pseudomonas	25	29.07
Escherichia coli	19	22.09
Staph. aureus	18	20.93
Proteus mirabilis	10	11.62
Coagulase staph negative	8	9.32
Klebsiella	6	6.97
Total	86	100

Table(2): Antibiotics test for Gram positive bacteria isolated from burn injury

No	Antibiotics	Resistance of s.aureus	Resistance of coagulase negative staphylococci
1	Amoxicillin/clavulanic acid	36%	28%
2	Amikacin	20%	S
3	Vancomycin	S	S
4	Imipenem	S	S
5	Cefepime	S	S

No	Antibiotics	P aeruoginosa	E.coli	*P. mirabilis*	*K. pneumonia*
1	Tobramycin	S	S	S	S
2	Amikacin	20%	23%	22%	23%
3	Cefotaxime	34%	26%	24%	36%
4	Imipenem	S	S	S	S
5	Cefepime	S	S	S	S

Table(3): Antibiotics test for Gram Negative bacteria isolated from burn injury

Table (4):Biofilm type for isolates bacterial

Isolates of bacteria	Strong of biofilm	Moderate of biofilm	Weak of biofilm	Total
p.aeruoginosa	16	8	1	25
E.coli	10	6	3	19
s.aureus	7	8	3	18
P. mirabilis	3	5	2	10
K. pneumonia	3	2	1	6
Coagulase-negative Staphylococci	2	4	2	8
Total	41	32	12	86

Conclusions

From the presented study, the author conclude that the most dominant pathogens in burn infection was *P.aeruginosa,E. coli*, *S.aureus* ,*P. mirabilis*, Coagulase-negative Staphylococci, *K. pneumonia*respectively . We also conclude that Imipenemand Cefepimeare an excellent choice for burn infection as a first choice, it has 100% against all the pathogens that isolated in the current study.

Conflict of interest : No conflict of interest

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Ethical Clearance: This study is ethically approved by the Institutional ethical

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